Chelonian Conservation And Biology

Vol. 18 No. 2 (2023) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.11(2).1232.1244

FORMULATION OF PLANT BASED SANITIZER AND ITS EVALUATION

Vikrant Verma[1], Parkhi Rastogi[2],Saurabh Singh[3],Aakash tyagi[4],Abhishek Sharma[4],Aasif Rao[4],Ganesh Prasad Mishra[1]

[1]Department of Pharmaceutical Chemistry, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut(U.P) INDIA -250005

[2]Department of Pharmacology, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut (U.P) INDIA -250005

[3]Department of Pharmaceutical Technology,Meerut Institute of Engineering and Technology Uttar Pradesh 250005

[4]Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut(U.P) INDIA -250005

Corresponding Author rastogi.parkhi@gmail.com

Abstract

Prepararion of alcohol free hand sanitizer with antimicrobial qualities is essential, particularly in pandemics where there is a low supply chain and high demand for ethanol and isopropanol. Preparing sanitizer using natural ingredients that are effective against a wide range of pathogens was the aim of this study. The outcomes demonstrated that prepared sanitizer were deemed to have acceptable organoleptic properties. The formulation pH was slightly acidic (about 3.8) because aloe vera, which has an acidic reputation, was present in large amounts (90% v/v). The results of the antimicrobial effectiveness test showed that the sanitizer that were prepared exhibited antimicrobial activities against a variety of gram-positive and gram-negative bacteria as well as the yeast Candida albicans. Twenty human volunteers participated in an acceptability study, which revealed that the hand sanitizer did not cause any symptoms of skin irritation. The prepared natural sanitizer may serve as a viable substitute for the widely used alcohol-based hand sanitizers according to this study.

Keywords: Alcohol, Sanitizer, Microorganisms, Infections, Essential Oils, Antimicrobial, Pandemics

Introduction

The development of SARS-CoV-2, a new virus, has created unprecedented challenges to global public health. Currently, COVID-19 efforts are primarily sympathetic and precautionary, with the goal of minimising transmission. Hand hygiene is a simple and efficient strategy for minimising illness transmission in public and clinical settings.[1]

Because of the corona virus disease pandemic of 2019 (COVID-19), the World Health Organization (WHO) has emphasised the necessity of hand cleanliness in preventing the transmission of the virus. Typically, alcohol-based hand sanitizer is included in hand hygiene facilities. Nonetheless, a high dose of alcohol or frequent use of alcohol-based hand sanitizers causes skin dryness, which leads to skin burning and increases the risk of skin infection. As a result, a natural bioactive ingredient originating from a readily available agricultural crop was chosen in place of alcohol-based hand sanitizer that is both safe and effective.[2]

The primary goal of the manufacture of poly herbal hand sanitizer is to encourage the hygiene of hand. In the reduction, control and prevention of infection which is acquired, it is a critical factor. The chain of microorganisms can be disrupted by the Hand sanitizer specifically bacteria and virus disseminating to all the other regions of body through hands. Alcohol is the most frequent active ingredient in hand sanitizer, while inactive compounds include thickening agents, humectants, and other additives. When compared to soaps, alcohol-based hand sanitizers are particularly powerful at killing microorganisms.[3]

Handwashing is one of several types of methods utilized to thwart the spread of this virus, as it has been with prior infectious infections. Hand sanitizers which are based on alcohol have developed a widespread substitute to old-fashioned washing of hands with water and soap in both community and clinical settings.[4]. Mandankumund et al. compared alcohol and non-alcohol based sanitizers and found that alcohol-based sanitizers are more effective than non-alcohol based sanitizers.[5].(Lee et al., 2020.)

To stop or reduce the transmission of coronavirus, people all over the world are utilising alcohol-based hand sanitizers on a large scale.The most widely used hand sanitizers available in market comprises hydrogen peroxide, isopropyl alcohols, and ethyl alcohol in various proportions. Mismanagement of regulations could result in toxicity in humans and the environment.[7]. Frequent usage of hand sanitizers has also been linked to an increased risk of various viral infections and antimicrobial resistance.[8]

Individually, people are seen consistently applying alcohol-based hand sanitizer with 60–70 percent alcohol, as recommended by the CDC (2020), whether meeting new people outside or returning home. Spraying individuals with substances like alcohol or chlorine has been documented to have a toxic influence on entities, irritation of skin and eye, bronchospasm through inhalation, and probably consequences in gastrointestinal tract which includes nausea and vomiting. When we use alcohol on your skin frequently, it thins and dries up our skin, making it

more prone to germs and illnesses. This raises the issue of alcohol-free or herbal-based hand sanitizers, which are increasingly available. Secondary metabolites including tannins, terpenoids, alkaloids, and flavonoids are abundant in medicinal plants because they have been shown to have stronger antibacterial activity.

AAPCC reported 10,824 hand sanitizer (alcoholic) exposure cases in children under the age of 12 in the first six months of 2020, and this report lead to the conclusion that poisoning of alcohol can be caused through a small quantity of alcohol which results in nausea, confusion, lethargy and in extreme cases, death and seizure of respiratory tract in children.[8][9]. When compared to alcoholic hand rubs, alcohol-free and herbal hand sanitizers are simple to make and provide greater effects. Hand sanitizers made from readily available herbal plants have precise efficiency in reducing microbial load from hands.

Figure 1 Types of formulations of sanitizer

According to a recent study, SARS-CoV-2 can be transmitted by aerosol and fomite, with the virus remaining alive and infectious in aerosols for hours and on surfaces for days, depending on the inoculum shed. As a result, it's critical to break the virus's transmission chain using tight infection control measures and contact isolation.[10][11]. To date, the most effective hand sanitizers are alcohol-based formulations comprising 62 percent 95 percent alcohol, which may denaturize microbial proteins and inactivate viruses. Due to the high alcohol content, toxicity to skin and fire hazards are the few problems and worries associated with this formulation in terms of fire dangers and skin toxicity. Various measures have been implemented to prevent virus transmission from person to person. Hand sanitation with hand sanitizer is one of the most essential strategies for reducing transmission. The demand for sanitizer is currently very high, as is the rate of sales. Herbal sanitizers utilize herbal antiviral plants for killing germs instead of alcohol.Alcohol-free hand sanitizer is less expensive than alcohol-based hand sanitizer.[12]. Sanitizer is quite vital when it comes to maintaining adequate hygiene. Because of the high demand for sanitizer during this epidemic, new brands of sanitizer have entered the market, however the effectiveness of sanitizers has long been questioned. Sanitizer will be ineffective if the correct amount of alcohol is not utilised.

Instead of using a synthetic preparation, the goal of this study is to formulate and evaluate a poly herbal hand sanitizer made from plants parts which are readily available. The rate at which sanitizer is produced cannot compete with the rate at which it is sold. As a result, a method for making a less expensive sanitizer using readily available components is aimed. In less than half an hour, we can make this sanitizer.Its production rate can keep up with market demand. This product can be purchased by persons of all socioeconomic levels. The frequent use of hand sanitizers is currently one of the most important strategies for dealing with COVID19 and reducing community spread of infections. However, due to the greater price, a big portion of the general public is unable to purchase them.As a result, a method for making inexpensive sanitizers using readily available herbal materials is described here. The cost of manufacturing 100 mL of sanitizer was estimated to be 15 rupees. The bulk production of this sanitizer could be very beneficial for the widespread use of sanitizers by the general public.

Demerits of alcohol sanitizers(Genesis of research)

Hand sanitizers containing alcohol can cause serious harm, including alcohol poisoning, especially in young infants.Alcohol-based formulations are combustible, and if they fall into the wrong hands, they can endanger a building's safety.If we need to sanitise your hands frequently, an alcohol-based hand sanitizer can irritate our skin and wipe away natural, protective oils that are necessary for proper skin maintenance. To kill germs, an alcohol-free hand sanitizer uses a different active component. When skin is exposed to alcohol, it becomes thinner and dryer, making it more susceptible to viruses and illnesses.A high dose of alcohol or frequent use of alcohol-based hand sanitizers causes skin dryness, which leads to skin burning and increases the risk of skin infection. As a result, a natural bioactive ingredient originating from a readily available agricultural crop was chosen in place of alcohol-based hand sanitizer that is both safe and effective.

Materials and method

Soxhelet apparatus was utilized in the present research for the manufacture of the sanitizer.Ethyl acetate was procured from Ceilo Petro Chemicals Haryana India,Jojoba oil was procured from Young Chemist Private Limited,Gujarat,Lemon oil was obtained from RK Worldinfocom Pvt ltd.Aloe vera was procured from RK Worldinfocom Pvt Ltd Haryana,Neem was procured from Isha Agro Developers pvt Limited,Maharastra,Ethyl alcohol was procured from ABC Traders,Bareilly,Polysorbate 20 was procured from Purenso Global ,Indore,Tree tea oil was procured from Merhaki Foods Nutrition Private Limited,Gujarat,Lemon oil was procured from RK Worldinfocom pvt ltd, Coriandrum sativum was procured from Nutrixia food,Maharashtra, Aegle marmelos was procured from Padmavathi Distributers,Andhra Pradesh,Tulsi leaf was procured from GPS Enterprises ,Tamil Nadu, Night blooming jasmine was procured from Apurba Mallick ,West Bengal,Guava leaves was procured from Greenwise ,West Bengal.

Materials

Table .1 Formulation table

S.No	Ingredients	Quantity(ml)
1.	Jojoba oil	1.3
2.	Lemon oil	1
3.	Tree tea oil	1.5
4.	Aloe vera	1
5.	Neem extract	1.5
6.	Polysorbate 20	5
7.	Aegle marmelos extract	1.2
8.	Tulsi	1
9.	Night blooming jasmine extract	1.3
10.	Guava leaves extract	1.2
11.	Water	Q.S

Methodology

Preparation of Hand Sanitizer

Fresh plant material was soxhelt extracted with 400 ml of water and ethyl acetate (temperature 100^{o} C) for 6 hours. The plant also extracted using a maceration process. All herbs are thoroughly cleansed to remove any particles or dust. Fresh plant material was dried in the shade and ground into a powder.

Alternatively the leaves were extracted using a maceration process with 70% ethanol and ethyl acetate and macerated for 3-4 days. The ingredients used to make the Non-alcohol based hand sanitizer, as well as their quantities, are utilized judiciously. After that, the mixture was filtered and evaporated to remove the alcohol. To achieve fluidity, the solvent-free combination was blended with distilled water. All herbs Tulsi, Aloevera,Jojoba oil, Night blooming jasmine,Coriandrum sativum,Ginger,Tea tree,Guava leaves,Neem are thoroughly cleansed to remove any particles or dust.

Herbal Lemon Essential Oil,Aegle marmelos and Allium sativum was added. Coriandrum sativum,GingerTea tree,Guava leaves,Neem were washed and put to water, which was then cooked until it achieved a syrupy consistency. After that, the syrup was filtered to remove any particles. After that, 7 mL of polysorbate-20 was added and thoroughly mixed. The above mixture is added to the syrup. Equal amounts of plant extracts made from a 10 gram of each plant were added.

Finally, other ingredients were added to the mixture and stirred thoroughly to produce a homogeneous liquid sanitizer. Sanitizer is prepared in a total volume of 100ml. The ingredients were purchased from the market. The amounts and their matching market prices are listed. The amount of ingredients used to produce the Sanitizer is then specified, and the prices are computed accordingly. The entire cost of producing the 100 mL sanitizer is also computed.

Strains of bacteria

The bacterial strains utilized in this investigation were isolated at the Kharvel Subharti College of Pharmacy. E. Coli and Bacillus subtilis ,Staphylococci aureus was utilized for the research.

Evaluation Parameters

1. Colour
2. Odour
3. pH
4. Tissue paper test
5. Wheat dough test[13]
6. Organoleptic characteristics, density, dispersive power, and homogeneity of the hand sanitizer were all examined.
7. The interview questionnaires were used to analyse the sensory effects of the hand sanitizers in 20 volunteers. The homogeneity test examines the stability during storage.

The microbiological load of hand sanitizer was compared to that of commercial hand sanitizer before and after it was applied to hands. Herbal hand sanitizer has the capability to reduce microbial load after application, with the highest reduction in microbial load being seen at 75% or even more.[14]

Organoleptic Test

In order to assess the texture, colour, and odour of the sanitizer the prepared samples were visually examined.

Evaluation of pH

A digital pH meter (Mettler Toledo pH meter,) was used to measure the formulated sanitizer pH. The pH readings are the average ± standard deviation (SD) of three independent experiments.[15]

Homogeneity test

The goal of the homogeneity test is to examine the sanitizer over time. By applying the sanitizer sample to a piece of glass or another appropriate transparent material, the homogeneity test of sanitizer was examined. The sanitizer dosage should indicate the homogeneous composition and the absence of coarse grains. The outcome demonstrated that coarse grains are not visible and the sanitizer is homogenous in form.

Examining the sanitizer's stability over storage is the goal of the homogeneity test. After the examination of sanitizer by placing it on two objective glasses, homogeneous form sanitizer was observed.[16]

Dispersive power

The dispersive power measurements are made in order to ascertain the sanitizer ability to distribute its dosage across the skin's surface and, consequently, to control the distribution of the sanitizer active ingredients. This has to do with how the dosage's active ingredients are distributed.

The test revealed that the sanitizer dosage's dispersive power complies with Indonesian National Standards (SNI). The dispersive power test was used to determine how well hand sanitizer spread across a surface. The dispersive power test also examines the sanitizer capacity to spread across the skin's surface, where it should be able to do so with ease when applied to the hands. The dispersive power value, satisfies the 5-7cm standard set by SNI No. 06-2588-1992.[2]

Irritability test

Five healthy volunteers were chosen for the irritability test.After applying the herbal hand sanitizer to the palm, the time was recorded. Checks were made for irritation, redness, dryness, and itching.

Evaporation rate

Five volunteers in good health were chosen. Their palm was treated with the herbal hand sanitizer, which was rubbed in. At that point, evaporation occurred, and it was recorded. Evaporation rate was less than one minute.

The hand sanitizer's physical stability

Weekly observations of the sanitizer's colour, odour, and pH were used to identify the physical changes. The sanitizer's pH was measured following preparation. Sanitizer had a pH of 3.8. The sanitizer colour, pH, and odour were assessed in each week. Three months were found to have passed with no changes to the pH, colour, or odour.

Assay for Antimicrobial Zone of Inhibition

Using a standard cup plate method, the antibacterial activity of herbal hand sanitizers with varying solvents was assessed against strains of both aerobic and anaerobic microorganisms. The nutrient agar medium served as the culture medium for this typical cup plate method. The pre-sterilized

petri plate was used for the antibacterial test. The petri plate was pre-sterilized by incubating it at 37˚C for 24 hours. Subsequently, the aseptic condition of the agar culture medium was applied evenly on the petri plate. Following spreading, the agar medium was covered with a second petri plate and refrigerated for a full day to solidify it. Subsequently, the plate was taken out, and the cup was created on these plates. The standard solution containing strains of E. Coli and Bacillus subtilis ,Staphylococci aureus was evenly distributed in an aseptic state on two specific plates.[17]

The bacterial suspensions, or inoculums, were prepared using Mueller-Hinton broth. Each microbe was grown on Mueller-Hinton agar medium and kept at 37 °C for the entire night in the incubator.

Table 2: Agar medium composition

S.NO	Ingredients	Quantity
1.	Peptic digest of animal tissue	5
2.	Sodium chloride	5
3.	Beef extract	1.5
4.	Yeast extract	1.5
5.	Agar	8.5

The zone of inhibition test was used to assess the prepared hand sanitizers antimicrobial efficacy against gram-negative and gram-positive bacterial strains, as well as C. Albicans yeast, in comparison to three commercially available hand sanitizers . Variable diameter well-defined zones of inhibition were seen.

USES OF DIFFERENT HERBS OR CHEMICALS

Tulsi-Antiviral

Aloevera- Antibacterial

Coriandrum sativum- Antimicrobial

Jojoba oil- Moisturizing

Tea tree- Antiviral

Ginger- Antiviral

Night blooming jasmine-Fragnance

Neem- Antiviral

Guava leaves- Anti-bacteria and Anti-viral

Polysorbate-20-Emulsifiers

Aegle marmelos- Antiviral

Allium sativum- Antibacterial, Antiviral, Antifungal and Antiprotozoal

Research on Skin Irritation (Acceptability Test)

A skin irritation study was conducted to evaluate the effectiveness of the sanitizer formulation, which was chosen based on the outcomes of the prior antimicrobial effectiveness test. Twenty volunteers participated in the research. Following an explanation of the study protocol and any potential adverse effects, consent forms were given to the volunteers to sign. Each volunteer's palm was sanitized with 1 ml of sanitizer for the assessment, and they were then left to stand for 5 minutes. For the purpose of the skin irritation study and acceptability test, each volunteer received a questionnaire. Clinical indicators of trauma, infection, or dermal abrasion were absent in every volunteer. The evaluation of the formulation was based on the features of the hand sanitizer, including appearance, smell, texture, burning or irritation sensation, and redness following application.

Results and discussion

The physical, antibacterial, and organoleptic qualities of the herbal hand sanitizer were assessed. The prepared herbal hand sanitizer formulation demonstrated good efficacy against E. Coli, Bacillus subtilis and Staphylococci aureus bacterial strains. It also possesses antibacterial, antimicrobial properties. The formulation was discovered to have a smooth texture, liquid consistency, and yellow colour with characteristics odour.

The purpose of the organoleptic test for hand sanitizer was to assess the prepared formulations physical appearance. After the prepared hand sanitizer were visually inspected for quality, the results showed the positive traits for the tested formulation. The sanitizer prepared had the good consistency, were clear and odorous and were simple to apply. A bubble-like appearance developed during the course of the overnight storage, but it vanished with a little shaking. The homogeneity of the prepared formulations prevented the hand sanitizer from displaying any coarse particles when it was spread on a transparent glass.

A digital pH meter was used to determine the hand sanitizer pH values. The purpose of the study was to determine whether or not prepared formulations could be neutralized. To prevent skin irritation and inflammation, the ideal pH range for a topical formulation is between 4.0 and 7.0, which is the wide range of the skin's pH . Prepared formulation had pH values of approximately

3.8, which indicates a slight acidity, according to pH measurements. This could be because aloe vera has a naturally acidic pH of 4.0–4.5 and added in the formulation .

Hand sanitizer shelf life

After three months of observation at various room temperatures, the sanitizer showed no signs of deterioration. The sanitizer was maintained at room temperature (27 ± 2°C) and high temperature (40 ± 2°C) and maintain a low temperature of 4 ± 2°C for three months in the refrigerator. A stable sanitizer was discovered in all of the three temperature variations. With regard to pH, colour, and odour, the physical stability was steady.

Acceptability Test for Skin irritation

The study on skin irritation involved 20 volunteers. Sanitizer was chosen for the acceptability test and skin irritation study based on the findings of the pH evaluation and its antimicrobial activity. The best hand sanitizers should smell good, feel comfortable to use, be non-sticky and easy to apply, and have strong antibacterial properties. According to the findings of the skin irritation study, the hand sanitizer was extremely well-tolerated by the participants and did not cause any irritation or skin redness. Out of the twenty volunteers, only five reported a slight itching sensation; four of them had eczema already and were undoubtedly showing redness. Sanitizer was thus applied to those four volunteers once more, and no negative effects were noted.

Antimicrobial Zone of Inhibition Assay:Commercially available hand sanitizers were compared to the produced hand sanitizers when tested using the zone of inhibition method against gram-positive and gram-negative bacterial strains as well as C. albicans yeast. Zones of inhibition with varying diameters and distinct borders were observed. Tables 3 and 4 show the antimicrobial activity results for each ingredient used in the sanitizer. Other sanitizers that were used produced less activity than the formulated herbal sanitizer, in comparison. The outcomes demonstrated that formulated herbal sanitizer is more effective and has strong antimicrobial activity when compared to other brands of hand sanitizer sold in stores.

Table No. 3: Hand sanitizer's Zone of Inhibition against Candida albicans when prepared and sold commercially

Code	Candida albicans
S1	12.5 ±1.31
F1	13.85±1.52

Table No. 4 Zone of inhibition for gram-positive and gram-negative bacteria in prepared, commercial hand sanitizers.

F.Code	E.coli	S.aureus	B.subtilus
S1	9.7 ±0.65	8.65±7.6	10.67±0.67
F1	11.87±0.65	10.92±0.55	13.28±0.55

Wheat Dough Test: It involved mixing one table spoon of wheat with some Sanitizer. Wheat will turn into dough if there is too much water Nothing in the way of dough formation was seen.

Conclusion

Hand hygiene is the primary goal in making a polyherbal hand sanitizer. It is an essential concept for the avoidance, management, and mitigation of any acquired infection. Generally speaking, hand sanitizers can break the chain of microorganisms and other bacteria that travel from our hands to various body parts. Maintaining good hand hygiene is crucial and one of the most important things to do when preparing food in homes, restaurants, and other day care facilities. Using hand sanitizer helps prevent side effects such as dermatitis, itching, and irritation.An novel technique is used for the formulation of effective hand sanitizer. More research is needed to evaluate the antibacterial effect and evaluate it against other hand sanitizer options. It may be concluded that herbal hand sanitizer has a significant bacterial effect on the specified microorganisms. Therefore, there is immense potential in establishing the use of antibacterial herbal products as a measure to control the multidrug resistant microbes as well as check their spread through hands from one geographical region to another.A significant amount of research has concentrated on maintaining hygiene by limiting the amount of pathogens that enter the body through the hands. Natural herbal hand sanitizer is affordable, efficient, safe for the environment, and biodegradable.

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